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Method Development and Validation for the Spectrophotometric Determination of Dihydroquercetin in Capsule Formulation

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ABSTRACT

Dihydroquercetin is a naturally occurring dihydroflavone found to be abundant in the woods of trees like larch. The other name for dihydroquercetin, which is commonly used, is Taxifolin. Dihydroflavone is a subclass of flavanols, which are a type of polyphenol. It is a bioactive component found in various food items, including herbal tea, fruits, and milk thistle. Dihydroquercetin is reported to have antioxidant properties and is used in the form of a health supplement. Numerous studies have reported the anti-inflammatory properties of taxifolin, its potential use in cancer treatment, and its application in the treatment of liver diseases, cardiovascular diseases, and neurodegenerative disorders. Additionally, it has been claimed to have antimicrobial actions. With the increase in potential activities for taxifolin, the possibilities for formulation development can be significantly enhanced. For the quantitative estimation of dihydroquercetin, the present study focused on the development of a simple, cost-effective method using UV and visible spectroscopy, which was validated according to the ICH guideline. The solvent used for the analysis is methanol 99% for both the UV and Visible (colorimetric) methods. Using the method, linearity for dihydroquercetin was found to exist in the concentration range of 1 to 5 µg/mL for the UV method and in the range of 80 to 120 µg/mL for the colorimetric methods. The parameters are also statistically validated to prove their significance. Various validation parameters, including accuracy, precision, linearity, range, and limits of measurement, such as limit of detection and limit of quantitation, were studied. The accuracy and precision of the

developed methods are tested in terms of percentage relative standard deviation for the observations, which were found to be less than 2%. The assay values for the finished formulations in capsule form were found to lie between the range of 99.7 and 99.8%w/w for UV and colorimetric methods. The correlation coefficient was calculated for the observations from the linearity plot and found to be near 1 for both methods. Thus, the developed methods can be employed as analytical tools to ensure the quality of marketed formulations.

1. Introduction

Dihydroquercetin (DHQ) is a bioactive dihydroflavone, classified as a flavanol, a type of polyphenol[1]. Chemically it is 2,3-dihydro-3,5,7-trihydroxy-2-(3,4-dihydroxyphenyl)-4*H*-1-benzopyran-4-one[2]. Flavanols are generally potent antioxidants that enhance immunity and also act as anti-inflammatory agents. DHQ is reported to have action against the SARS-CoV-2 virus by diminishing the infection[3]. Dihydroquercetin, also commonly referred to as taxifolin, has been demonstrated to be a potent antioxidant[4, 5] and is being formulated as a nutritional supplement. It is reported to have inhibitory actions against microbial infections[6], malignancy[7], dementia[8], and hepatic and cardiovascular diseases[9]. Dihydroquercetin is formulated as a food supplement or used in food and beverages to improve stability by preventing the oxidation of the food. From the literature survey, it is clear that there is no spectroscopical analytical method reported for estimating the DHQ in formulation. The methods reported employ sophisticated instrumental methods for quantitation of DHQ. In a research for analyzing DHQ in plant extracts of Pinus densiflora HPLC method was employed[10]. In another study, UHPLC-MS/MS method for the determination of DHQ in nanodispersion formulation in the rat plasma after liquid-liquid extraction, was reported[11]. High - performance liquid chromatography (HPLC - UV Vis) was employed for the quantitation of DHQ in food supplements.[12]. Simultaneous quantification of Taxifolin and Taxifolin-3-O-rhamnoside was reported also using HPLC[13]. Several other studies reported methods are based on UPLC-QTOF-MS, LC-MS/MS[14]. Such methods involve usage for costlier solvents while they are time consuming From the literature, it is evident that no simple analytical methods have been reported for estimating DHQ in formulations. In the present work, an attempt is made to estimate DHQ in the finished formulation (capsule) using accurate, precise, and time-efficient spectrophotometric UV and Visible colorimetric methods. Spectroscopy cost-effective, economic, and accurate technique of analysis[15,16]. The developed methods showed good linearity obeying Beer-Lambert's law[17]. The methods are validated by studying various parameters like linearity, range, precision, accuracy, LOD and LOQ[18].

2. Materials and Methods

2.1 Instrument

The instrument employed for analysis was a Shimadzu double spectrophotometer, UV-1900 series, with a slit width of 1.0 nm, sample holders made of quartz with a 1 cm path length, and a threshold of 0.0010000.

2.2 Materials

2.2.1 For UV method

Capsule formulation with a label claim of dihydroquercetin as 25mg and methanol of 99%

2.2.2 For Visible/colorimetric method

Capsule formulation with a label claim of dihydroquercetin at 25 mg, methanol, ferric chloride solu-

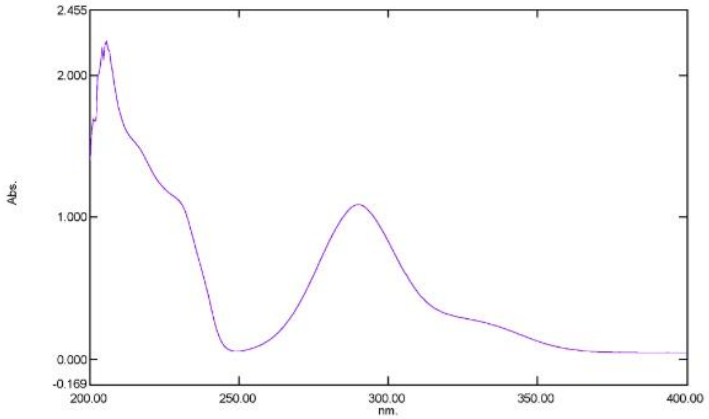

Figure 1 – *Spectrum of DHQ in standard (UV)*

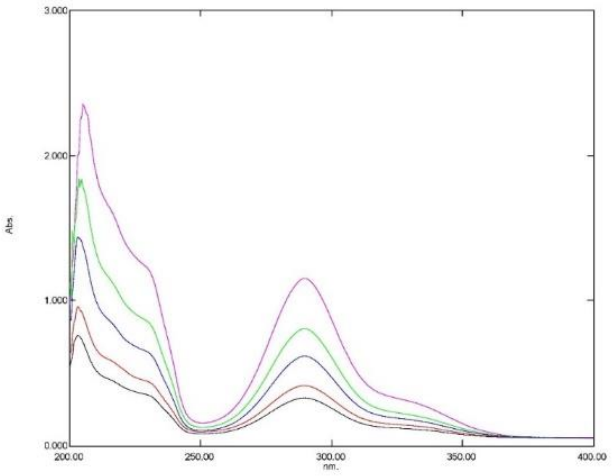

Figure 2 – *Overlain spectrum of DHQ in a series of concentrations (UV)*

tion, and distilled water.

2.3 Method

2.3.1 UV method

2.3.1.1 Standard stock solution

10mg of dihydroquercetin was weighed accurately, and its solution was prepared by using 10 mL of methanol as solvent to give a concentration of 1 mg/mL.

2.3.1.2 Working standard solution

From the standard stock solution of dihydroquercetin, 0.1 mL was pipetted out using a micropipette and diluted to 10 mL with methanol to achieve a concentration of 10 µg/mL.

2.3.1.3 Wavelength maximum

The working standard solution of DHQ was diluted by pipetting out 5 mL and diluting to 10 mL with methanol. This solution was scanned in the

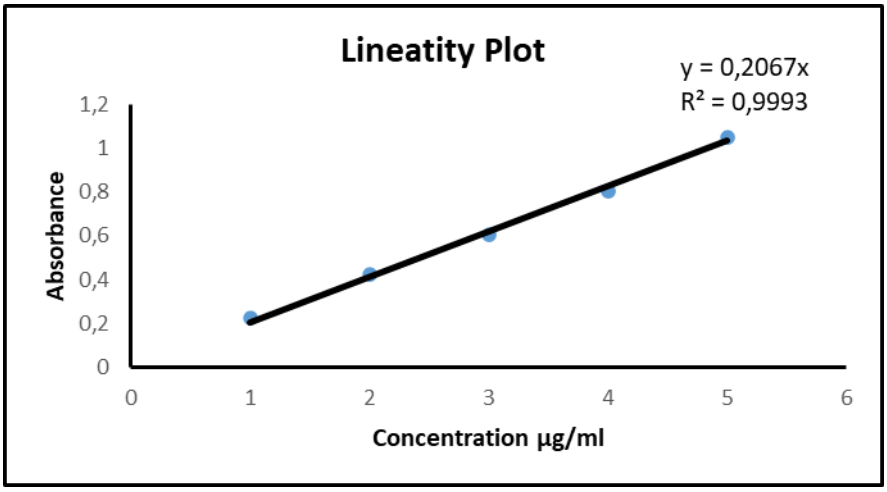

Figure 3 *Linearity plot (UV method)*

UV wavelength range of 200-400 nm using a UV spectrophotometer using methanol as solvent. From the spectrum recorded, the wavelength maximum of dihydroquercetin was found to exist at 289nm. The spectrum of dihydroquercetin is shown in Figure 1.

2.3.1.4 Linearity Range:

To determine the linearity range for dihydroquercetin, the standard stock solution was diluted to a series of concentrations ranging from 1 to 5 µg/mL with 99% methanol as the solvent. The absorbance of each concentration was recorded and a calibration curve was constructed. The overlaid spectrum of dihydroquercetin showing linearity, is shown in Figure 2.

The calibration plot is shown in Figure 3. It was found that DHQ showed good linearity in the concentration range of 1 to 5 µg/mL with a correlation coefficient r= 0.9961

2.3.2 Colorimetric method

2.3.2.1 Ferric chloride solution

1.61g of solid ferric chloride was weighed and dissolved in an aliquot of distilled water. One drop of Hydrochloric acid (36%) solution was added, and then it was diluted to 100 mL with distilled water.

2.3.2.2 Standard stock solution

The standard stock solution was prepared by dissolving 10 mg of DHQ in 10 mL of methanol to give a final concentration of 1mg/mL or 1000 µg/mL.

2.3.2.3 Working standard solution

From the standard stock solution, an aliquot was taken, treated with ferric chloride solution, and then diluted with distilled water to the required volume to achieve the desired concentration.

2.3.2.4 Wavelength maximum

The standard solution was treated with a ferric chloride solution, kept for 10 minutes, and diluted to 10 mL with distilled water. This solution was scanned in the visible wavelength range of 400-800 nm, in the spectrophotometer using ferric chloride solution as blank. From the spectrum recorded, the wavelength maximum of dihydroquercetin was found to be at 672nm.

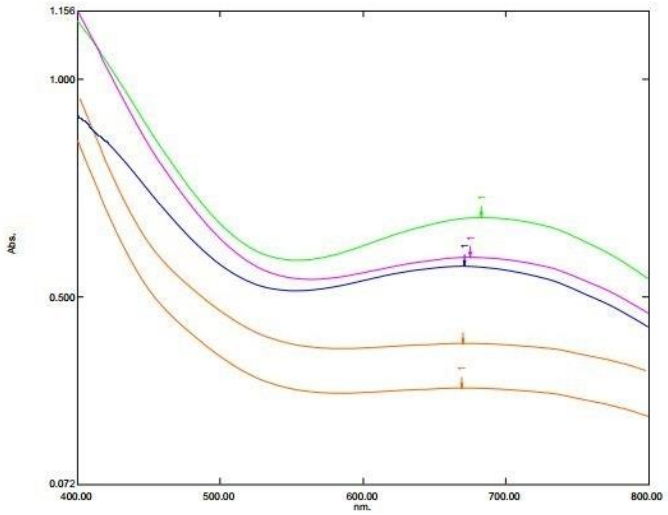

Figure 4: *Overlain spectra for linearity (Colorimetric method)*

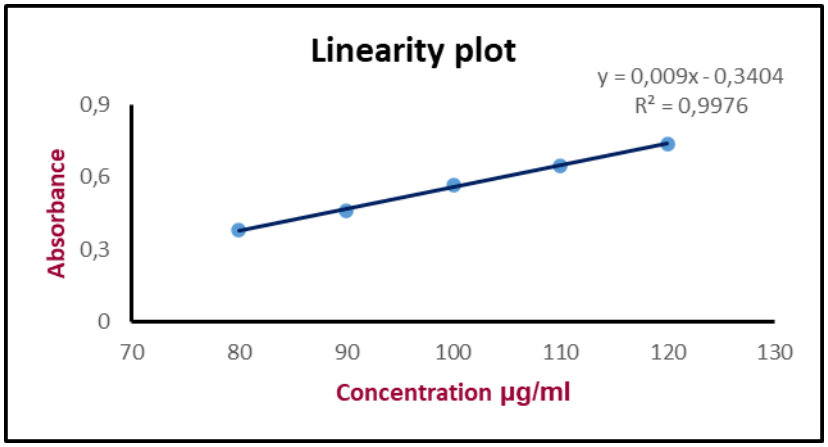

Figure 5: *Linearity plot (Colorimetric method)*

2.3.2.5 Linearity range

The standard solution was taken in different aliquots, treated with a ferric chloride solution, allowed to stand for 10 minutes, and then diluted with distilled water to prepare a series of concentrations ranging from 80 µg/mL to 120 µg/mL. The overlain spectrum showing the linearity is depicted in Figure 4.

The absorbance of the solutions at 672nm was noted, and a linearity plot was constructed by plotting concentration versus absorbance, as shown in Figure 5. It was found that the DHQ exhibits linearity within the concentration range of 80 µg/mL to 120 µg/mL, with a correlation coefficient value r= 0.9976.

2.4 Assay

2.4.1 Sample preparation for UV method

Twenty capsules were weighed together, and their

combined weight was noted. The contents of the capsules were emptied, and the weight of the empty capsules was found out. From the difference, the weight of the capsule content was calculated. From the capsule content, a weight of the powder equivalent to 10mg of DHQ was found and transferred to a standard volumetric flask. The drug was dissolved using methanol as a solvent. The solution of the capsule content was decolorized by adding activated animal charcoal. The solution was treated with a small quantity of animal charcoal, shaken for a few minutes until the color completely disappeared, filtered off. The resulting solution was made up to a volume of 10 mL with methanol. From this solution, 3 mL was pipetted out and diluted to 10 mL with methanol. The concentration of the resulting solution was 3μg/mL (middle concentration within the linearity range).

2.4.2 Sample preparation for colorimetric method

Twenty capsules were weighed together, and their combined weight was recorded. The content of the capsules was emptied, and the weight of the empty capsule was determined. From this, the weight of the capsule content was calculated. From the capsule content, aweight of the powder equivalent to 10mg of DHQ was found and dissolved in methanol. The solution was treated with a small quantity of animal charcoal, shaken well for 5 minutes. The resulting solution was made up to the volume of 10mL with methanol. The solution was filtered using Whatmann filter paper, and the filtrate was collected. An aliquot of this filtrate was treated with ferric chloride solution, allowed to stand for 10 minutes, and diluted with distilled water to give a final concentration of 100μg/mL.

2.4.3 Assay calculation

The sample solution was diluted in replicates (n = 6) to the required concentrations of 3 μg/mL and 100 μg/mL for the UV and colorimetric methods, respectively. The analysis was repeated by measuring the absorbance at the wavelength maximum, determined for both UV and colorimetric methods. The amount of drug present in the sample was calculated for each observation recorded, and the average of the values was calculated. The amount of drug present in the pharmaceutical dosage form was calculated using the formula:

Amount of drug present

$$\frac{A_T \times C_S \times D_f \times W_1}{A_S \times W_2}$$

Where,
A_T = absorbance of test
A_s = absorbance of standard
C_S = concentration of standard
D_F = dilution factor
W_1 = average weight
W_2 = weight of sample taken
The results are expressed in terms of the percentage label claim.

2.5 Validation Parameters for UV and Colorimetric Methods

2.5.1 Precision

The precision of the developed methods was assessed by taking aliquots of the working standard and repeating the analysis on the same day and on different days, where the drug is treated and diluted in the same manner as mentioned in the solution preparation for the respective methods. With the results, the % relative standard deviation for the data was calculated, and it was found to be less than 2%, showing statistical significance.

2.5.2 Accuracy

Accuracy was studied in terms of percentage recovery. A DHQ solution of known concentration was added to the pre-analyzed sample solution, diluted to the required volume with methanol, and analyzed

in replicate. For colorimetry, the solutions prepared by adding the standard to the pre-analyzed sample, is treated with ferric chloride reagent and the observations in terms of absorbance were recorded. The results were expressed in terms of percentage recovery, which were found to be between 100.5% and 100.6% w/w.

2.5.3 Limits of measurement

Limits of measurement were determined in terms of limit of detection (LOD) and limit of quantitation (LOQ), calculated with the standard deviation of the data and the slope of the linearity plot.

$$LOQ = \frac{\text{Standard deviation} \times 10}{\text{Slope}}$$

$$LOD = \frac{\text{Standard deviation} \times 3.3}{\text{Slope}}$$

3. Results and Discussion

The developed methods were employed to quantitatively estimate the amount of dihydroquercetin in pharmaceutical dosage form using UV and Visible spectroscopy.

The amount of drug present in the capsule formulation was estimated by comparing the absorbance of the sample with that of the standard, and the percentage label claim was found to be in the range of 99.7 to 99.9 %w/w for both UV and Colorimetric methods, which is in good agreement with the labelled amount.

The precision of the developed methods was assessed by replicate analysis of standard and sample solutions. The results are expressed in terms of percentage relative standard deviation, which was found to be 0.0234 for the UV method and 0.0381 for the colorimetry method, which are less than 2%. Thus the developed methods were said to be precise.

The accuracy of the method was studied by performing the recovery studies. The percentage recovery was found to be in the range of 100.5 to 100.6 % for UV method with %RSD of 0.0574 (<2%) and in the range of 99.8 to 100.3% for colorimetric method with %RSD of 0.2553 (<2%), which lies within the acceptable limit, and so the methods were found to be accurate.

The limits of measurement, including the limit of detection and the limit of quantitation, were found to be 0.016 and 0.048 μg/mL for the UV method and 0.048 and 1.267 μg/mL for the colorimetric method, respectively. The results for method evaluation are tabulated in Table 1.

4. Summary and conclusion

The present work involved the development of UV and visible/colorimetric spectrophotometric methods for the determination of dihydroquercetin (taxifolin) in pharmaceutical formulations.

The results of the assay, in terms of percentage label claim, show that the amounts of drugs were in good agreement with the label claim of the formulation.

The percentage relative standard deviation values for system precision were found to be not more than 2% in both UV and visible methods. The accuracy of the method was evaluated through recovery studies. The percentage recovery values for dihydroquercetin were within the acceptable limit, indicating the accuracy of the methods and suggesting that the methods are free from interferences. The limits of measurement, including the limit of detection and the limit of quantitation, were also studied and reported with the UV method showing lower values.

From the values calculated for the assay and validation parameters, it is suggested that both methods can be used as a routine analytical procedure for the quality control of DHQ in pharmaceutical dosage forms. Since the methods involve UV instrumentation, the time consumption for performing the analysis is shorter compared to HPLC method or LC-MS/MS. In addition, solvents for the UV method are more economical. Due to the increasing exploration of the significant pharmacological effects of taxifolin/DHQ and the development of various dosage forms of the

Table 1. Characteristics and validation parameters of the developed methods

PARAMETERS	OBSERVATIONS	
	UV METHOD	**VISIBLE METHOD**
Wavelength maximum (λmax)	289nm	672nm
Linearity range (µg/ml)	1 to 5	80 to 120
Correlation coefficient	0.9961	0.9976
Slope	0.2067	0.009
Intercept	0	0.3404
Label claim (mg)	25	25
Mean % Label claim (%w/w)	99.77	99.73
System Precision **% R.S.D (NMT 2%)**	0.0234	0.0381
Mean % Recovery **% R.S.D (NMT 2%)**	100.53 0.0574	100.02 0.2553
Limit of detection (µg/mL)	0.016	0.418
Limit of quantitation (µg/mL)	0.048	1.267

drug the methods described in this study can be effectively employed for qualifying the formulations, facilitating the batch-to-batch analysis within a short span of time.

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